COMPOSITIONS AND METHODS FOR SYNTHESIZING NUCLEIC ACIDS

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] The present application claims the benefit of the filing dates of U.S. Provisional Appl. Nos. 60/408,609, filed September 5, 2002, and 60/427,867, filed November 19, 2002, the disclosures of both of which are incorporated herein by reference in their entireties.

STATEMENT REGARDING FEDERALLY SPONSORED RESEARCH OR DEVELOPMENT

Not applicable.

~~REFERENCE TO SEQUENCE LISTING/TABLE/COMPUTER PROGRAM LISTING APPENDIX (submitted on a compact disc and an incorporation by reference of the material on the compact disc)~~

~~Not applicable.~~

BACKGROUND OF THE INVENTION

Field of the Invention

[0002] This invention relates to methods and materials useful for nucleic acid synthesis (e.g., polymerase chain reaction-based nucleic acid synthesis).

Related Art

[0003] DNA polymerases (DNAPs) synthesize DNA molecules that are complementary to all or a portion of a nucleic acid template (preferably a DNA template). Upon hybridization of a primer to a DNA template to form a primed template, DNA polymerases can add nucleotides to the 3' hydroxy end

AccuPrime Taq DNA polymerase were used in a typical 50 µl reaction. Primer sets used in development of AccuPrime Taq DNA polymerase system and its applications are listed in Table 2.

TABLE 2

p32D9 149 bp	5' 3'
Forward primer:	ATC CCC CAC CCC CGC ACC (SEQ ID NO:12)
Reverse primer:	GGG CGC GAG ATG GGC TGC (SEQ ID NO:13)
Pr1.2 235 bp	5' 3'
Forward primer:	TTG GAG GGG TGG GTG AGT CAA G (SEQ ID NO:14)
Reverse primer:	GGA GGG GTG GGG GTT AAT GGT TA (SEQ ID NO:15)
Pr1.3 265 bp	5' 3'
Forward primer:	GCA TCT GGG GCC TGG GAT TTA G (SEQ ID NO:16)
Reverse primer:	TAC AAG GCA GGC ATC ATG ACT CAC G (SEQ ID NO:17)
p53 gene 504 bp	5' 3'
Forward primer:	TGC CGT CCC AAG CAA TGG ATT T (SEQ ID NO:18)
Reverse primer:	CAG GAG AGA TGC TGA GGG TGT GGA (SEQ ID NO:19)
c-myc gene 822 bp	5' 3'
Forward primer:	CGG TCC ACA AGC TCT CCA CTT G (SEQ ID NO:20)
Reverse primer:	CTG TTT GAC AAA CCG CAT CCT TG[[c-]] (SEQ ID NO:21)
c-myc gene 1069 bp	5' 3'
Forward primer:	GGT TTT CGG GGC TTT ATC TAA CTC (SEQ ID NO:22)
Reverse primer:	GCC TAC CCA ACA CCA CGT CCT (SEQ ID NO:23)
p53 gene 1587 bp	5' 3'
Forward primer:	GCT GCC GTG TTC CAG TTG CTT TAT C (SEQ ID NO:24)

Reverse primer: GCA GCT CGT GGT GAG GCT CCC (SEQ ID NO:25)
p53 gene 1996 bp 5' 3'
Forward primer: CCT TGG CTT TTG AAA ATA AGC TCC TGA (SEQ ID NO:26)
Reverse primer: GCA GCT CGT GGT GAG GCT CCC (SEQ ID NO:27)
p53 gene 2108 bp 5' 3'
Forward primer: GCA GAG ACC TGT GGG AAG CGA AAA (SEQ ID NO:28)
Reverse primer: GAG AGC TGT GGC AAG CAG GGG A (SEQ ID NO:29)
Rhodopsin gene 3047 bp 5' 3'
Forward primer: GCC CTA ACT TCT ACG TGC CCT TCT (SEQ ID NO:30)
Reverse primer: [[\]]AGG CTT CCA GCG CAC GTC ATT (SEQ ID NO:31)
p53 gene 4356 bp 5' 3'
Forward primer: CCC CTC CTG GCC CCT GTC AT (SEQ ID NO:32)
Reverse primer: GTT AGA TGA CTT TGC CCA ACT GTA GGG (SEQ ID NO:33)

[0212] Thermocycling was conducted using either the Perkin Elmer GeneAmp PCR System 9600 or the Perkin Elmer GeneAmp PCR System 2400.

[0213] Standard PCR program:

94°C 2 minutes

35 cycles of

94°C	15 seconds
55°C - 60°C	30 seconds (5 degrees below Tm)
68°C	1 min/kb

Hold at 4°C

[0214] Following the completion of thermocycling, PCR amplification products were mixed with 5 ml of 10x BlueJuice and aliquot (20%, or 10 µl, of total reaction volume per each lane) were analyzed on 0.8% -1.5% agarose gel electrophoresis with an ethidium bromide concentration of 0.5 µg/ml

94°C	15 seconds
55°C-60°C	30 seconds (5 degrees below Tm)
68°C	1 min/kb

Hold at 4°C

[0223] Multiplex PCR. Random designs of primer sets from different genes were selected for multiplex PCR. To determine the optimal conditions, titrations were conducted involving all practical aspects of a standard PCR reaction such as:

a) DNA template – using 100 ng, 200 ng, and 500 ng.

b) Enzyme units – with 2 units, 5 units, and 10 units.

c) dNTP – focusing on 0.1 mM, 0.2 mM, and 0.4 mM final concentrations.

d) $MgCl_2$ – centering on, 1.2, 1.5, 1.8, 2, and 2.5 mM final concentrations.

e) Single Stranded Binding Protein concentration – 200, 400, 600, and 800 ng.

[0224] PCR reactions were prepared on ice in the standard format using 100 ng of K562 genotyping DNA as a template and 2 - 5 units of enzyme in addition to the obvious substitution of each of the variables as outlined above. The primer sets used in multiplex PCR are listed in Table 3.

TABLE 3

#1	Tms1 – 44	5' 3'
	Forward primer:	GGC TGG AGT GCA GTG GTG CAA T (SEQ ID NO:34)
	Reverse primer:	GGC AGA GGC TAC AGT GAG CCA A (SEQ ID NO:35)
#2	Thal – 57	5' 3'
	Forward primer:	GGG CAG AGC CAT CTA TTG CTT ACA (SEQ ID NO:36)
	Reverse primer:	GGT TGC TAG TGA ACA CAG TTG TGT CA (SEQ ID NO:37)

#3	Hba2 – 67	5'	3'
	Forward primer:	GCA CTC TTC TGG TCC CCA CAG A (SEQ ID NO:38)	
	Reverse primer:	TTG GTC TTG TCG GCA GGA GAC A (SEQ ID NO:39)	
#4	Rgr – 74	5'	3'
	Forward primer:	CCC ACG ATC AAT GCC ATC AAC T (SEQ ID NO:40)	
	Reverse primer:	CGG TGA GAG GCA CTG CCA GAT T (SEQ ID NO:41)	
#5	B-glo-thal – 84	5'	3'
	Forward primer:	GCT CGC TTT CTT GCT GTC CAA T (SEQ ID NO:42)	
	Reverse primer:	GCC CTT CAT AAT ATC CCC CAG TTT (SEQ ID NO:43)	
#6	c-myc – 100	5'	3'
	Forward primer:	GTC CTT CCC CCG CTG GAA AC (SEQ ID NO:44)	
	Reverse primer:	GCA GCA GAG ATC ATC GCG CC (SEQ ID NO:45)	
#7	Zip – 116	5'	3'
	Forward primer:	GTG GGG GTG CTG GGA GTT TGT (SEQ ID NO:46)	
	Reverse primer:	TCG GAC AGA AAC ATG GGT CTG AA (SEQ ID NO:47)	
#8	Csh1 – 135	5'	3'
	Forward primer:	GGT GCT CAG AAC CCC CAC AAT C (SEQ ID NO:48)	
	Reverse primer:	CCT ACC GAC CCC ATT CCA CTC T (SEQ ID NO:49)	
#9	Sub – 153	5'	3'
	Forward primer:	CAC AGA TTT CCA AGG ATG CGC TG (SEQ ID NO:50)	
	Reverse primer: CGT GCT CTG TTC CAG ACT TG (SEQ ID NO:51)		
#10	Svmt – 170	5'	3'

	Forward primer:	CGT CTG GCG ATT GCT CCA AAT G (SEQ ID NO:52)
	Reverse primer:	GGG CAG TTG TGA TCC ATG AGA A (SEQ ID NO:53)
#11	Olf – 183	5' 3'
	Forward primer:	GGC TTG CAC CAG CTT AGG AAA G (SEQ ID NO:54)
	Reverse primer:	CGT TAG GCA TAA TCA GTG GGA TAG T (SEQ ID NO:55)
#12	P53 – 193	5' 3'
	Forward primer:	GCC TCT GAT TCC TCA CTG ATT GCT CT (SEQ ID NO:56)
	Reverse primer:	TGT CAA CCA CCC TTA ACC CCT CC (SEQ ID NO:57)
#13	Pr 1.2 – 237	5' 3'
	Forward primer:	TTG GAG GGG TGG GTG AGT CAA G (SEQ ID NO:58)
	Reverse primer:	GGA GGG GTG GGG GTT AAT GGT TA (SEQ ID NO:59)
#14	Hmk – 243	5' 3'
	Forward primer:	GGA ACA AGA CAC GGC TGG GTT (SEQ ID NO:60)
	Reverse primer:	AGC AAG GCA GGG CAG GCA A (SEQ ID NO:61)
#15	Rhod – 273	5' 3'
	Forward primer:	CGG TCC CAT TCT CAG GGA ATC T (SEQ ID NO:62)
	Reverse primer:	GCC CAG AGG AAG AAG AAG GAA A (SEQ ID NO:63)
#16	Caaf1 – 300	5' 3'
	Forward primer:	GCC CCC ACC CAG GTT GGT TTC TA (SEQ ID NO:64)
	Reverse primer:	ATG CCT TCA TCT GGC TCA GTG A (SEQ ID NO:65)
#17	P-450 B – 319	5' 3'
	Forward primer:	GCT CAG CAT GGT GGT GGC ATA A (SEQ ID NO:66)
	Reverse primer:	CCT CAT ACC TTC CCC CCC ATT (SEQ ID NO:67)
#18	S-100 – 360	5' 3'

	Forward primer: GAC TAC TCT AGC GAC TGT CCA TCT C (SEQ ID NO:68)	
	Reverse primer: GAC AGC CAC CAG ATC CAA TC (SEQ ID NO:69)	
#19	B-cone - 432	5' 3'
	Forward primer: GGC AGC TTT CAT GGG CAC TGT (SEQ ID NO:70)	
	Reverse primer: GAC AGG GCT GGA CTG ACA TTT G (SEQ ID NO:71)	
#20	Hbg – 469	5' 3'
	Forward primer: CTG CTG AAA GAG ATG CGG TGG (SEQ ID NO:72)	
	Reverse primer: AGG AAA ACA GCC CAA GGG ACA G (SEQ ID NO:73)	

[0225] Standard program for multiplex PCR reactions

94°C 2 minutes

35 cycles

94°C	15 seconds
60°C	30 seconds (5 degrees below Tm)
68°C	1 min/kb

Hold at 4°C

[0226] The PCR products were then analyzed on a 3% horizontal agarose gel with an ethidium bromide concentration of 0.5 μg/ml premixed in 0.5 x TBE. Comparisons were made visually for specificity and yield between the different samples.

[0227] High throughput PCR. Accuprime Taq DNA polymerase was compared with Platinum™ Taq DNA polymerase (Invitrogen Corp.) to examine for improvement in high throughput screening. Standard PCR was performed for 18 cycles of amplification using 2 Units of Accuprime Taq DNA polymerase and 2 Units of Platinum Taq DNA polymerase.

[0228] Transformed cells plated on X-gal/IPTG/Amp plates containing the pUC19 plasmid DNA insert were used as plasmid template for high throughput screening. Mutant colonies were selected with a sterile pipette tip and mixed in the standard PCR reactions. PCR cycling parameters were 94°C

expression in a particular host, depending on the desired expression level. Thus, a single rare codon or a larger percentage (e.g., 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, and 100%) of rare codons can be optimized in an SSB gene.

[0371] Attempts to express and purify archaeal SSBs in *E. coli* have met with problems associated with codon bias. For example, SSBs from *Methanococcus jannachii* (MjaSSB) and *Sulfolobus solfataricus* (SsoSSB) are expressed at relatively low levels in BL21(DE3) cells. In addition, SsoSSB co-purifies with shorter peptides most likely truncated proteins arising from premature termination. Present in high enough amounts, the shorter peptides can negate the SsoSSB-mediated PCR improvement.

[0372] The MjaSSB and SsoSSB genes use codons that are rarely used in *E. coli*. For example, in the native MjaSSB and SsoSSB genes AGA or AGG call for arginine, ATA calls for isoleucine, and CTA calls for leucine (Tables 16 and 17; rare codons are underlined). Many of these rare codons occur in tandem pairs or triplets, which may be responsible for the low expression level and/or truncated peptide contaminants.

TABLE 16

Sequence of the native SsoSSB gene
atg gaa gaa aaa gta ggt aat CTA aaa cca aat atg gaa agc gta
aat gta acc gta AGA gtt ttg gaa gca agc gaa gca AGA caa ATA
cag aca aag aac ggt gtt AGA aca atc agt gag gct att gtt gga
gat gaa acg gga AGA gta aag tta aca tta tgg gga aaa cat gca
ggt agt ATA aaa gaa ggt caa gtg gta aag ATA gaa aac gcg tgg
acc acc gct ttt aag ggt caa gta cag tta aat gct gga agc aaa
act aag ATA gct gaa gct tca gaa gat gga ttt cca gaa tca tct
caa ATA cca gaa aat aca cca aca gct cct cag caa atg cgt gga
gga gga AGA gga ttc cgc ggt ggg gga AGA AGG tat gga AGA AGA
ggt ggt AGA AGA caa gaa aac gaa gaa ggt gaa gag gag tga
(SEQ ID NO:84)

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att aaa gat gaa aac att gaa gct cca gag tat gag CTA
aaa tat tgc aaa att gaa gat att tat aat AGA gat gtt
gac tgg aac gat ATA aat tta ATA gct caa gtt gtt gag
gat tat gga gtt aat gaa att gaa ttt gaa gat aag gtt
AGA aaa gta AGA aat tta ttg tta gaa gat gga act gga
AGA ATA AGG ttg agt tta tgg gat gat ttg gct gaa ATA
gag att aaa gaa gga gat att gta gaa att tta cat gcc
tat gct aag gag AGG gga gat tat ATA gat ttg gtt att
gga aaa tat gga AGA ATA att ATA aat cca gaa ggg gtt
gaa ATA aaa acc aat AGA aag ttt ATA gca gat att gaa
gac gga gaa act gtt gaa gtt AGA ggg gct gta gtt aag
ATA ttg agt gac act ctc ttt ctt tat tta tgc cca aat
tgt AGA aag AGG gtt gta gag att gat gga att tat aac
tgc cct att tgt gga gat gtt gag cca gaa gag att tta
AGA ttg aat ttt gtt gta gat gat ggg act gga act tta
tta tgt AGG gct tat gat AGA AGA gtt gag aag atg tta
aaa atg aat AGG gag gag tta aag aac CTA act ATA gaa
atg gtg gaa gat gaa ATA tta ggg gaa gag ttt gtt ttg
tat gga aat gtt AGA gta gag aat gat gaa tta att atg
gtt gtt AGA AGA gtt aat gat gta gat gtt gag aaa gaa
ATA AGA ATA ttg gag gaa atg gaa taa (SEQ ID NO:85)
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[0373] Codon optimization of SsoSSB. To test whether low expression of SsoSSB was related to codon bias, the native gene was transformed into BL21 CodonPlus with supplementary tRNA genes for Arg (AGA, AGG), Ile (AUA) and Leu (CUA) rare codons (Stratagene). When expressed in this host, a SsoSSB was produced at higher levels (compare lanes 12 and lane 13 in Figure 43), and less truncated peptide was present after purification (compare Figures 30 & 31 with Figure 28).

[0374] We replaced the rare codons in the SsoSSB gene with codons common in *E. coli* using "synthetic gene" technology (Stemmer, W. P. et al. (1995) *Gene* 164:49-53). Thus, AGA and AGG were replaced by CGG, CGT, CGA

or CGC; ATA was replaced by ATT or ATC; and CTA was replaced by CTT, CTG, or CTA (Table 18; optimized codons are underlined and in bold italics).

TABLE 18

Codon optimized recombinant SsoSSB gene
atg gaa gaa aaa gta ggt aat ***ctg*** aaa cca aat atg gaa agc gta aat gta acc gta ***cga*** gtt ttg gaa gca agc gaa gca ***cgt*** caa ***atc*** cag aca aag aac ggt gtt ***cgg*** aca atc agt gag gct att gtt gga gat gaa acg gga ***cga*** gta aag tta aca tta tgg gga aaa cat gca ggt agt ***atc*** aaa gaa ggt caa gtg gta aag ***att*** gaa aac gcg tgg acc acc gct ttt aag ggt caa gta cag tta aat gct gga agc aaa act aag ***atc*** gct gaa gct tca gaa gat gga ttt cca gaa tca tct caa ***att*** cca gaa aat aca cca aca gct cct cag caa atg cgt gga gga gga ***cgc*** gga ttc cgc ggt ggg gga ***cgt cgg*** tat gga ***cga cgt*** ggt ggt ***cgc cgg*** caa gaa aac gaa gaa ggt gaa gag gag tga (SEQ ID NO:86)

[0375] To make a codon optimized SsoSSB gene, 21 overlapping primers were used (Table 19). The primers were mixed together in equal amounts (approximately 4.5 uM) in a PCR reaction without template DNA. PCR was performed using Taq Hi-FI Supermix (Invitrogen Corp.). A thermocycler was programed for 20 cycles of denaturation at 94°C for 30 sec, annealing at 55°C for 30 sec, and elongation at 72°C for 30. An aliquot of this PCR reaction (2 µl or 1/25 of the volume) was added to a second PCR reaction with two 2 anchor primers that anneal at the 5' and 3' ends of the reassembled gene (Table 19). These primers also add a NdeI site to the 5' end and a BamHI site to the 3' end of the gene. After 2 more rounds of PCR using the parameters set out above, a discrete product of about 450 base pairs was obtained. The product was excised from an electrophoresis gel, purified, and cloned into pET21a vector at the NdeI and BamHI sites in the multi-cloning site. The resulting clone was sequenced to confirm the sequence.

TABLE 19

Forward Primers	
Sso F1	ATGGAAGAAA AAGTAGGTAA TCTGAAACCA AATATGGAAA GC (SEQ ID NO:87)
Sso F2	GTAAATGTAA CCGTACGAGT TTTGGAAGCA AGCGAAGCAC GT (SEQ ID NO:88)
Sso F3	CAAATCCAGA CAAAGAACGG TGTTCGGACA ATCAGTGAGG CT (SEQ ID NO:89)
Sso F4	ATTGTTGGAG ATGAAACGGG ACGAGTAAAG TTAACATTAT GG (SEQ ID NO:90)
Sso F5	GGAAAACATG CAGGTAGTAT CAAAGAAGGT AAGTGGTAAA G (SEQ ID NO:91)
Sso F6	ATTGAAAACG CGTGGACCAC CGCTTTTAAG GGTCAAGTAC AG (SEQ ID NO:92)
Sso F7	TTAAATGCTG GAAGCAAAAC TAAGATCGCT GAAGCTTCAG AA (SEQ ID NO:93)
Sso F8	GATGGATTTC CAGAATCATC TCAAATTCCA GAAAATACAC CA (SEQ ID NO:94)
Sso F9	ACAGCTCCTC AGCAAATGCG TGGAGGAGGA CGCGGATTCC GC (SEQ ID NO:95)
Sso F10	GGTGGGGGAC GTCGGTATGG ACGACGTGGT GGTCGCCGGC AA (SEQ ID NO:96)
Sso F11	GAAAACGAAG AAGGTGAAGA GGAGTGA (SEQ ID NO:97)
Reverse Primers	
Sso R1	TCACTCCTCT TCACCTTCTT CGTTTTCTTG CCGGCGACCA CC (SEQ ID NO:98)

Sso R2	ACGTCGTCCA TACCGACGTC CCCCACCGCG GAATCCGCGT CC (SEQ ID NO:99)
Sso R3	TCCTCCACGC ATTTGCTGAG GAGCTGTTGG TGTATTTTCT GG (SEQ ID NO:100)
Sso R4	AATTTGAGAT GATTCTGGAA ATCCATCTTC TGAAGCTTCA GC (SEQ ID NO:101)
Sso R5	GATCTTAGTT TTGCTTCCAG CATTTAACTG TACTTGACCC TT (SEQ ID NO:102)
Sso R6	AAAAGCGGTG GTCCACGCGT TTTCAATCTT TACCACTTGA CC (SEQ ID NO:103)
Sso R7	TTCTTTGATA CTACCTGCAT GTTTTCCCCA TAATGTTAAC TT (SEQ ID NO:104)
Sso R8	TACTCGTCCC GTTTCATCTC CAACAATAGC CTCACTGATT GT (SEQ ID NO:105)
Sso R9	CCGAACACCG TTCTTTGTCT GGATTTGACG TGCTTCGCTT GC (SEQ ID NO:106)
Sso R10	TTCCAAAACT CGTACGGTTA CATTTACGCT TTCCATATTT GG (SEQ ID NO:107)
Sso R11	TTTCAGATTA CCTACTTTTT CTTCCAT (SEQ ID NO:108)
Anchor primers	
Sso F NdeI	AATTCATATG GAAGAAAAAGT AGGT (SEQ ID NO:109)
Sso R BamHI	GGAAGGATCC TCACTCCTCTT CACCTTC (SEQ ID NO:110)

CGT	***ATC***	aca	aac	tgt	***CGC***	gtt	aag	acg	ttt	tat	gat
CGT	gaa	gga	aat	aaa	***CGG***	act	gat	tta	gtt	gcc	aca
tta	gaa	aca	gaa	gtt	att	aaa	gat	gaa	aac	att	gaa
gct	cca	gag	tat	gag	***CTG***	aaa	tat	tgc	aaa	att	gaa
gat	att	tat	aat	***CGC***	gat	gtt	gac	tgg	aac	gat	***ATA***
aat	tta	***ATC***	gct	caa	gtt	gtt	gag	gat	tat	gga	gtt
aat	gaa	att	gaa	ttt	gaa	gat	aag	gtt	***CGT***	aaa	gta
CGC	aat	tta	ttg	tta	gaa	gat	gga	act	gga	***CGT***	***ATT***
CGG	ttg	agt	tta	tgg	gat	gat	ttg	gct	gaa	***ATT***	gag
att	aaa	gaa	gga	gat	att	gta	gaa	att	tta	cat	gcc
tat	gct	aag	gag	***CGG***	gga	gat	tat	***ATC***	gat	ttg	gtt
att	gga	aaa	tat	gga	***CGA***	***ATT***	att	***ATC***	aat	cca	gaa
ggg	gtt	gaa	***ATC***	aaa	acc	aat	***CGT***	aag	ttt	***ATT***	gca
gat	att	gaa	gac	gga	gaa	act	gtt	gaa	gtt	***CGC***	ggg
gct	gta	gtt	aag	***ATC***	ttg	agt	gac	act	ctc	ttt	ctt
tat	tta	tgc	cca	aat	tgt	***CGT***	aag	***CGG***	gtt	gta	gag
att	gat	gga	att	tat	aac	tgc	cct	att	tgt	gga	gat
gtt	gag	cca	gaa	gag	att	tta	***CGA***	ttg	aat	ttt	gtt
gta	gat	gat	ggg	act	gga	act	tta	tta	tgt	***CGG***	gct
tat	gat	***CGC***	***CGT***	gtt	gag	aag	atg	tta	aaa	atg	aat
CGG	gag	gag	tta	aag	aac	***CTT***	act	***ATC***	gaa	atg	gtg
gaa	gat	gaa	***ATT***	tta	ggg	gaa	gag	ttt	gtt	ttg	tat
gga	aat	gtt	***CGA***	gta	gag	aat	gat	gaa	tta	att	atg
gtt	gtt	***CGT***	***CGC***	gtt	aat	gat	gta	gat	gtt	gag	aaa
gaa	***ATT***	***CGT***	***ATC***	ttg	gag	gaa	atg	gaa	taa	(SEQ	ID
NO:111)											

[0379] The primers identified in Table 21 are used to replace the rare codons in the MjaSSB gene with codons common in *E. coli* using "synthetic gene" technology, as was done for the SsoSSB gene. The forward and reverse primers are about 60 nucleotide long and overlapping at least 15 nucleotides with the neighboring primers.

TABLE 21

Forward Primers		
Mja	F1	ATGGTAGGAG ATTATGAACG TTTTAAACAA CTCAAAAAAA AGGTTGCTGA AGCATTGAAT (SEQ ID NO:112)
Mja	F2	GATAAAAAAA TTGAAGAAAA CGGAGGAATC ATTTTGAAAG ATGCTGCATT AATGATGATT (SEQ ID NO:113)
Mja	F3	AAAAATGATG AAGAATTTTT AATTAGTGAT ATTGAAGAGG GACAGATTGG CGTTGAGATC (SEQ ID NO:114)
Mja	F4	AAAACATTCA AACGGCGCGA TGGGAGTTTA GGGAAATACA AACGAATTAC AATTGCGGAT (SEQ ID NO:115)
Mja	F5	GACGATTTGG CTGAATTAGA TGTAAAAGTT GGAGATGTTA TTAAAATTGA ACGCGCACGG (SEQ ID NO:116)
Mja	F6	AGTTCAACAT CTGAAACTAA GATTAAAAAA TTAGAAAACT ATGAAGGAGA ACTTCCAGAG (SEQ ID NO:117)
Mja	F7	AGTCCTGGAA TGACAGCAAC ATTTGAAGGA GAAGTTATCT CAGCTCTTCC AATCAAAGAA (SEQ ID NO:118)
Mja	F8	TTAAAATCAT TTATTGTTCG CGATGAGACA GGAAGTATTC GCGTTACCTT ATGGGATAAT (SEQ ID NO:119)
Mja	F9	TACGTTCGTG TTCGGGGCTA TATCCGGGAA GGTTATTATG GGGGTTTAGA ATGCACCGCA (SEQ ID NO:120)
Mja	F10	AAAATAGAGA GTGAAGAAGT AAATATTGAG GATTTAACAA AATATGAAGA TGGAGAACTG (SEQ ID NO:121)
Mja	F11	AGTAATAAAA AAAGCGTAGA TTTGGATGGA GAGATTGCAA AGGTTCAAGA TATTATCTTA (SEQ ID NO:122)
Mja	F12	TTTTGGCGGG GAAAAACTGC TTTATTGGAA AATATCAAAG AAGGGGACTT AGTTCGTATC (SEQ ID NO:123)
Mja	F13	CGTGAAGGAA ATAAACGGAC TGATTTAGTT GCCACATTAG AAACAGAAGT TATTAAAGAT (SEQ ID NO:124)
Mja	F14	AAATATTGCA AAATTGAAGA TATTTATAAT CGCGATGTTG ACTGGAACGA TATAAATTTA (SEQ ID NO:125)
Mja	F15	AATGAAATTG AATTTGAAGA TAAGGTTCGT AAAGTACGCA ATTTATTGTT AGAAGATGGA (SEQ ID NO:126)
Mja	F16	GATTTGGCTG AAATTGAGAT TAAAGAAGGA GATATTGTAG AAATTTTACA TGCCTATGCT (SEQ ID NO:127)
Mja	F17	ATTGGAAAAT ATGGACGAAT TATTATCAAT CCAGAAGGGG TTGAAATCAA AACCAATCGT (SEQ ID NO:128)
Mja	F18	ACTGTTGAAG TTCGCGGGGC TGTAGTTAAG ATCTTGAGTG ACACTCTCTT TCTTTATTTA (SEQ ID NO:129)
Mja	F19	ATTGATGGAA TTTATAACTG CCCTATTTGT GGAGATGTTG AGCCAGAAGA GATTTTACGA (SEQ ID NO:130)

Mja	F20	ACTTTATTAT GTCGGGCTTA TGATCGCCGT GTTGAGAAGA TGTTAAAAAT GAATCGGGAG (SEQ ID NO:131)
Mja	F21	GAAGATGAAA TTTTAGGGGA AGAGTTTGTT TTGTATGGAA ATGTTCGAGT AGAGAATGAT (SEQ ID NO:132)
Mja	F22	GATGTAGATG TTGAGAAAGA AATTCGTATC TTGGAGGAAA TGGAATAA (SEQ ID NO:133)
Reverse Primers		
Mja	R1	TTCAATTTTT TTATCAATCA TCCGATCTAA TTCCTCCTCA CTAATATTCA ATGCTTCAGC (SEQ ID NO:134)
Mja	R2	TTCTTCATCA TTTTTTTCTT CTCCATAAAC TCCATGTTCT TTTGCAATCA TCATTAATGC (SEQ ID NO:135)
Mja	R3	CCGTTTGAAT GTTTTGATTT CAGAGATATC AGTAATAACT CCAGTGATCT CAACGCCAAT (SEQ ID NO:136)
Mja	R4	TTCAGCCAAA TCGTCCCATA AAGTCATACG AATAGTTCCT GACTTATCCG CAATTGTAAT (SEQ ID NO:137)
Mja	R5	TTCAGATGTT GAACTCAACT CTAAATTATT TCGCCATTTA CGTGCCCGTG CGCGTTCAAT (SEQ ID NO:138)
Mja	R6	TGTCATTCCA GGACTCAGCT CACCAATATT GTAGGTATCT TTAATCTCTG GAAGTTCTCC (SEQ ID NO:139)
Mja	R7	AATAAATGAT TTTAATTTTC CAATACTACC ATCAGCACGT TTAAATTCTT TGATTGGAAG (SEQ ID NO:140)
Mja	R8	CCGAACACGA ACGTAATCTC CACGACCAAC ATCGATATCT GTAAGATTAT CCCATAAGGT (SEQ ID NO:141)
Mja	R9	TTCACTCTCT ATTTTTTCTC CTTTTTTTAA AATCTCTACA TAATTTGCGG TGCATTCTAA (SEQ ID NO:142)
Mja	R10	GCTTTTTTTA TTACTGATGG CAATAACTCG ACCTTTAACA CTCACCAGTT CTCCATCTTC (SEQ ID NO:143)
Mja	R11	TTTTCCCCGC CAAAATGAAA CACGAACTCG ACCAGTGCCG TTATCTAAGA TAATATCTTG (SEQ ID NO:144)
Mja	R12	TTTATTTCCT TCACGATCAT AAAACGTCTT AACGCGACAG TTTGTGATAC GAACTAAGTC (SEQ ID NO:145)
Mja	R13	AATTTTGCAA TATTTCAGCT CATACTCTGG AGCTTCAATG TTTTCATCTT TAATAACTTC (SEQ ID NO:146)
Mja	R14	AAATTCAATT TCATTAACTC CATAATCCTC AACAACTTGA GCGATTAAAT TTATATCGTT (SEQ ID NO:147)
Mja	R15	AATTTCAGCC AAATCATCCC ATAAACTCAA CCGAATACGT CCAGTTCCAT CTTCTAACAA (SEQ ID NO:148)
Mja	R16	TCCATATTTT CCAATAACCA AATCGATATA ATCTCCCCGC TCCTTAGCAT AGGCATGTAA (SEQ ID NO:149)
Mja	R17	GCGAACTTCA ACAGTTTCTC CGTCTTCAAT ATCTGCAATA AACTTACGAT TGGTTTTGAT (SEQ ID NO:150)

Mja R18	ATAAATTCCA TCAATCTCTA CAACCCGCTT ACGACAATTT GGGCATAAAT AAAGAAAGAG (SEQ ID NO:151)
Mja R19	CCGACATAAT AAAGTTCCAG TCCCATCATC TACAACAAAA TTCAATCGTA AAATCTCTTC (SEQ ID NO:152)
Mja R20	TAAAATTTCA TCTTCCACCA TTTCGATAGT AAGGTTCTTT AACTCCTCCC GATTCATTTT (SEQ ID NO:153)
Mja R21	CTCAACATCT ACATCATTAA CGCGACGAAC AACCATAATT AATTCATCAT TCTCTACTCG (SEQ ID NO:154)
Mja R22	TTATTCCATT TCCTCCAAGA TACGAATTTC TTT (SEQ ID NO:155)
Anchor primers	
Mja F	GCTGCCATGG TAGGAGATTA TGAACGTTTT AAACAAC (SEQ ID NO:156)
Mja R	GCTCCTCGAG TTATTCCATT TCCTCCAAGA TACG (SEQ ID NO:157)